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Prognostic Significance of Glutathione S-Transferase π Expression and Subcellular Localization in Human Gliomas[1]

Francis Ali-Osman,[2] Janet M. Brunner, Tezer M. Kutluk, and Kenneth Hess

Section of Molecular Therapeutics, Department of Experimental Pediatrics [F. A-O., T. M. K.], Section of Neuropathology, Division of Pathology [J. M. B.], Department of Biomathematics [K. H.], and the Brain Tumor Center, University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

ABSTRACT

The glutathione *S*-transferase (GST)-π gene is overexpressed in many human cancers and preneoplastic lesions and is associated with failure of cancer chemotherapy and poor patient survival. Although GST-π overexpression in tumors of the central nervous system has been observed, the prognostic and/or clinical relevance of this overexpression has to date, not been investigated. In this study, we analyzed the level of GST-π expression and its subcellular localization in 60 primary gliomas and correlated the results with tumor histology, patient age, and patient survival. We observed a strong positive correlation between the level of GST-π expression and tumor grade and between the presence of GST-π in glioma cell nuclei and patient age. Univariate and multivariate Cox regression analyses and Kaplan-Meier curves showed the level of GST-π expression and its nuclear localization to be inversely correlated with patient survival. Relative risk for death of patients with high *versus* low tumor GST-π expression was 3.2 ($P = 0.0069$) by univariate analysis and 2.6 ($P = 0.036$) by multivariate analysis. The relative risk of death associated with the presence of nuclear GST-π in glioma cells was 3.9 ($P = 0.0001$) by univariate analysis and 4.4 ($P < 0.0001$) by multivariate analysis. These data indicate that high GST-π expression in tumor cells and the presence of the GST-π protein in tumor cell nuclei are associated with clinically more aggressive gliomas and are strong predictors of poor patient survival.

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To whom requests for reprints should be addressed, at Section of Molecular Therapeutics, Department of Experimental Pediatrics, Box 87, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: (713) 792-3495; Fax:

INTRODUCTION

Malignant gliomas are the most common and therapeutically least responsive tumors of the central nervous system, and there are, generally, no long-term survivors among patients with high-grade gliomas (1). Despite efforts to identify structural and functional abnormalities in oncogenes, tumor suppressor genes, and other cancer- and cell growth-related genes that may be involved in the development and progression of gliomas (2), no major molecular correlates of glioma progression and/or patient survival have, as yet, been identified. For other tumors, however, a number of genes and proteins have been shown to have significant prognostic value. Among these are the genes encoding the GSTs.[3]

GSTs are a family of dimeric proteins best known for their function as phase II enzymes in which they catalyze the conjugation of GSH with electrophilic compounds (3–5). They are highly heterogeneous proteins encoded by genes that are structurally different and are located on different chromosomes (5–7). Of the four major classes of human soluble GSTs (α, μ, π, and θ) identified to date, GST-π has been most widely associated with human cancer (3, 8–10). A large body of evidence (11–29) has accumulated over the past decade demonstrating GST-π gene overexpression to be associated with the early stages of carcinogenesis, such as in the liver, the uterine cervix, and the stomach, and to be a common feature of many human tumors, including brain tumors, malignant melanoma, acute leukemia, non-Hodgkin's lymphoma, and carcinomas of the bladder, breast, colon, head and neck, kidney, liver, lung, pancreas, ovary, stomach, testes, and uterine cervix. For several of these tumors, little to no expression of the GST-π protein is present in their corresponding normal tissues (19, 30–32). A particularly significant and consistent finding in many of these studies is that of a strong correlation between high tumor GST-π levels and failure of patients to respond to chemotherapy and low patient survival rates (33–42).

A small number of reports (17, 18, 23, 40) have examined the expression of GSTs in human primary brain tumors and shown that in malignant gliomas, the predominantly expressed GST is of the pi class. In an earlier study (40), we reported that elevated GST-π protein levels correlated with 2-chloroethylnitrosourea resistance in human glioma cell lines. Recently (41), our laboratory isolated three closely related, full-length GST-π cDNA variants from λgt 11 libraries of human glioma cells as well as the gene of one of these variants, *hGST-P1*C*,[4] which is

[3] The abbreviations used are: GST, glutathione *S*-transferase; GSH, glutathione; CI, confidence interval.

[4] H-W. Lo and F. Ali-Osman. Genomic cloning and expression in Cos 1 cells of a variant human π class glutathione *S*-transferase gene containing functional retinoic acid response elements. J. Biol. Chem., in press.

expressed at a higher frequency in gliomas than in normal cells and tissues. Together, these findings provide conclusive molecular evidence that the human *GST-π* gene locus is polymorphic. Despite these advances in our understanding of the molecular nature of the *GST-π* gene in gliomas, the interrelationship between *GST-π* gene expression, patient survival, and other parameters of prognostic significance in gliomas are unknown. Thus, the clinical significance of GST-π overexpression in gliomas and its possible bearing on patient survival remain undetermined. The goal of this study was to address these important issues by determining the level of GST-π expression and its subcellular localization in primary human gliomas and to correlate these findings with important clinicopathological parameters and patient survival. The results of these analyses should help determine whether the level or pattern of GST-π expression has prognostic value in human gliomas.

MATERIALS AND METHODS

Antibodies, Biochemicals, and Other Reagents. Rabbit polyclonal antibody against human placental *GST-π* was obtained from Biotrin, Inc. (Dublin, Ireland) and was tested at various dilutions to determine the optimum concentration required for reproducible immunohistological staining with minimum background staining. Mouse anti-rabbit antibody and nonimmunized rabbit IgG were purchased from Becton Dickinson (Palo Alto, CA). The same batch of antibodies were used throughout the study. All other reagents, unless otherwise stated, were purchased from Sigma Chemical Co. (St. Louis, MO).

Patients and Tumors. All patients in the study had surgery at the University of Texas M. D. Anderson Cancer Center. The study had received prior approval of the Institutional Review Board. Surgery and diagnosis were made independent of the study.

Specimens were processed by fixation for 6–24 h in neutral 10% formalin and stained with H&E. After histological diagnosis and grading of the tumors by a neuropathologist (J. M. B.), additional 4-μm-thick sections were cut from each specimen for GST-π staining and coded to conceal patient identity. Upon completion of the GST-π immunocytochemical analyses, the results were provided to the biostatistician (K. H.), who obtained from patients' hospital records the relevant clinical and histological information required for the statistical correlations. Reference points for survival were the date of surgery, date of last follow-up, or date of death. Tumors were categorized into one of the following histological groups: glioblastoma multiforme, anaplastic astrocytoma and "other" gliomas (astrocytomas, oligodendrogliomas, and oligo-astrocytomas). Such a categorization has been shown previously to be prognostically relevant (42).

Immunocytochemistry for GST-π Expression. Immunocytochemical staining for GST-π was performed essentially as we had described previously (43) with only minor modifications. Paraffin sections were prewarmed to 60°C, deparaffinized in two exchanges of xylene, rinsed in decreasing ethanol concentrations (100–70%), and rehydrated in PBS. Endogenous peroxidase was inactivated with 0.3% H_2O_2 in methanol, and the slides were incubated overnight with a polyclonal rabbit anti-human GST-π antibody at a 1:500 dilution. The slides were rinsed with four exchanges of cold (4°C) PBS and incubated with an avidin-conjugated mouse anti-rabbit antibody for 30 min. After further rising with cold PBS, as described above, the slides were treated with a solution of biotinylated peroxidase (Vector Laboratories, Burlingame, CA) and developed with 0.05% diaminobenzidine and 0.01% H_2O_2 in 50 mM Tris·HCl buffer (pH 7.5). Nonimmunized rabbit IgG was used as a negative control for the GST-π antibody, and the MGR-1 glioblastoma cell line was used as a positive control for GST-π staining.

Table 1 Distribution of gliomas according to histological category and level of GST-π expression

Histology	*n*	Level of GST-π expression[a]		
		High	Moderate	Low
Glioblastoma multiforme	33	15 (46%)	9 (27%)	9 (27%)
Anaplastic astrocytoma	13	4 (31%)	7 (54%)	2 (15%)
Other gliomas	15	4 (27%)	4 (27%)	7 (46%)
All gliomas	61	23 (38%)	20 (33%)	18 (29%)

[a] $P = 0.16$ by exact χ^2 test.

Quantitation of the Level of GST-π Expression and Evaluation of Its Subcellular Localization. Following immunocytochemical staining, the level of GST-π expression in each specimen was determined by scoring the staining intensity of 600 cells (200 cells in each of three different microscopic fields selected randomly at ×200). GST-π staining intensity was assessed as low, moderate, or high, based on the cytoplasmic staining intensity of 70% or greater of tumor cells. Subcellular GST-π expression was characterized as the presence or absence of GST-π immunoreactivity in the cytoplasm and/or nuclei of tumor cells in the same microscopic field evaluated for the level of GST-π expression. The GST-π staining characteristics of other nontumor cells, e.g., reactive astrocytes, endothelial cells, and infiltrating lymphocytes, were noted but not used in the evaluation of GST-π expression in the tumors. To validate the immunocytochemical staining procedure, 22 specimens were randomly selected and independently evaluated for GST-π staining by a neuropathologist and compared with those of the investigator performing the overall evaluation.

Statistical Analysis. The relationship between GST-π expression and histology was determined using the Kruskal-Wallis test (exact version). The presence of nuclear GST-π in glioma cells as a function of age was determined by probability estimates. The correlation of the level of GST-π expression and of the presence or absence of nuclear GST-π in glioma cells with patient survival was determined by both univariate and multivariate analyses, using the Cox proportional hazard regression model (44). Survival estimates were computed and plotted by the Kaplan-Meier method (45). Covariates in the multivariate analyses were age (continuous) and histology.

RESULTS

Tumor and Patient Characteristics. Tumors from 61 patients were examined in this study. The distribution of these specimens according to histology is shown in Table 1. Thirty-three (54%) of the 61 specimens were glioblastoma multiforme,

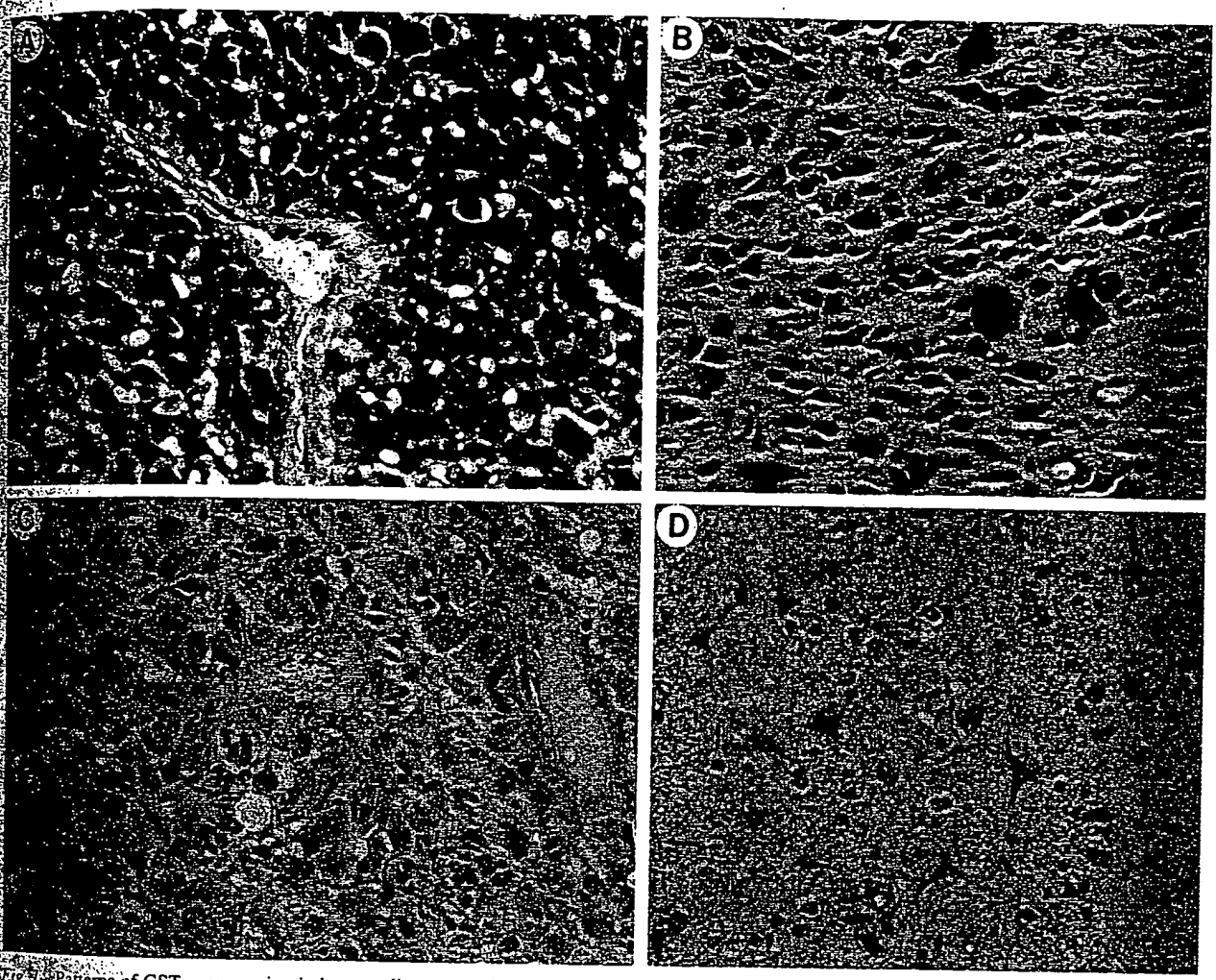

Fig. 1. Patterns of GST-π expression in human gliomas. *A*, glioblastoma with a high level of GST-π staining in both cytoplasm and cell nuclei. *B*, glioblastoma with a high level of GST-π staining but in which GST-π is present in the cytoplasm and absent in cell nuclei. *C*, glioblastoma with a moderate level of GST-π expression. *D*, low-grade astrocytoma showing "reactive" astrocytes with strong cytoplasmic and negative nuclear GST-π expression. Note the lack of GST-π staining in the capillary endothelial cells in *A* and *C*.

13 (21%) were anaplastic astrocytomas, and 15 (25%) were other (lower grade) gliomas. Of the 61 patients, 59 were newly diagnosed and had received no therapy prior to the analysis for GST-π expression; the remaining two had recurrent glioblastomas.

Pattern and Heterogeneity of GST-π Staining. GST-π expression in each tumor was categorized as low, moderate, or high, depending on whether GST-π immunoreactivity was weak or absent, intermediately strong, or very strong. Because the cytoplasm was always positive, regardless of the presence or absence of nuclear GST-π, the level of GST-π expression was based on cytoplasmic staining. Fig. 1 shows the typical patterns and the level of heterogeneity of GST-π staining in representative gliomas. Fig. 1, *A* and *B*, both glioblastomas, represent tumors with high GST-π expression. Fig. 1*C* shows a glioblastoma with a low level of GST-π expression, whereas Fig. 1*D* shows a low-grade astrocytoma with some GST-π-positive "reactive" astrocytes. The cells of the tumors in Fig. 1, *A* and *B*, demonstrate the two basic patterns of subcellular GST-π localization observed in glioma cells in this study. In Fig. 1*A*, both the cytoplasm and nuclei of the tumor cells stained strongly for GST-π. In contrast, in Fig. 1*B*, GST-π staining was present only in the cytoplasm of tumor cells and was absent in cell nuclei. We did not observe any tumor in which cell nuclei stained positively for GST-π, whereas the cytoplasm was negative. Generally, when both nuclei and cytoplasm were GST-π positive, the two subcellular compartments were similar with respect to the intensity of GST-π staining. In the majority of gliomas, the level of tumor cell GST-π immunoreactivity was uniform; however, occasionally, a significant degree of intercellular heterogeneity in GST-π staining was observed within a tumor. Note the negative GST-π staining of the capillary endothelial cells in Fig. 1, *A* and *C*. Normal nonreactive astrocytes, tumor-infiltrating lymphocytes, and areas of micro necrosis were also generally negative for GST-π. Nuclear GST-π was always absent in reactive astrocytes, even when the cytoplasm was strongly positive (Fig. 1*D*).

Table 2 Relationship between level of GST-π expression in gliomas and patient age and histology

GST-π expression	Age (yr)			
	Minimum	Median	Maximum	*n*
All gliomas				
Low	24	48	75	18
Moderate	15	46	69	20
High	24	58	71	23
P (Kruskal-Wallis) = 0.27				
Glioblastoma multiforme				
Low	30	50	75	9
Moderate	34	57	69	9
High	49	60	71	15
P (Kruskal-Wallis) = 0.16				
Anaplastic astrocytoma				
Low	24	26	27	2
Moderate	15	42	66	7
High	24	36	52	4
P (Kruskal-Wallis) = 0.23				
Other gliomas				
Low	28	47	68	7
Moderate	38	40	40	4
High	29	41	46	4
P (Kruskal-Wallis) = 0.78				

Table 3 Distribution of gliomas according to nuclear GST-π expression

	n	No. (%) of tumors with nuclear GST-π	
Level of GST-π expression			
Low	18	2 (11%)	0.000[a]
Moderate	20	11 (55%)	
High	23	17 (74%)	
Histology			
Glioblastoma multiforme	33	18 (55%)	0.63[b]
Anaplastic astrocytoma	13	6 (46%)	
Other gliomas	15	6 (40%)	
Age (yr)			
15–39	18	4 (22%)	0.0024[c]
40–49	12	6 (50%)	
50–59	12	5 (42%)	
60–75	19	15 (79%)	

[a] χ^2.
[b] Exact χ^2 test.
[c] Kruskal-Wallis.

Table 4 Univariate and multivariate Cox proportional hazard regression analyses of the relationship between the level of GST-π expression in gliomas, presence of GST-π in glioma cell nuclei, tumor histology, and patient age

The multivariate model was adjusted for age (continuous) and histology and accounted for 51% of variation in survival time.

	Relative risk	95% CI	*P*
Univariate analyses			
GST-π level			
High *vs.* low	3.2	(1.38, 7.5)	0.0069
Moderate *vs.* low	2.6	(1.07, 6.3)	0.035
Nuclear GST-π			
Present *vs.* absent	3.9	(2.0, 7.6)	<0.0001
Multivariate analyses			
GST-π level			
High *vs.* low	2.6	(1.1, 6.2)	0.036
Moderate *vs.* low	2.4	(1.0, 5.9)	0.051
Nuclear GST-π			
Present *vs.* absent	4.4	(2.1, 9.2)	0.0001

Correlation of GST-π Expression Level with Tumor Histology and Patient Age. The distribution of the histological categories of gliomas according to their level of GST-π expression is illustrated in Table 1. Within the different categories, a statistically significant association was observed between the proportion of tumors expressing high or low GST-π and the histological grade of the tumor. Thus, of the glioblastomas, 46% had high and 27% had low GST-π expression compared with 31 and 15%, respectively, of anaplastic astrocytoma and 27 and 46%, respectively, of "other" gliomas. Among the histological categories, however, although the levels of GST-π staining increased with glioma grade, the correlation was not statistically significant ($P = 0.16$).

The results of the statistical analyses of the correlation of GST-π expression with age are shown in Table 2. The median ages of patients with high, moderate, and low GST-π-expressing tumors was 58, 46, and 48 years, respectively. There was a modest trend toward higher GST-π expression in gliomas of older patients; however, the correlation was not statistically significant for all glioma patients ($P = 0.27$), for patients with glioblastoma multiforme ($P = 0.16$), or for anaplastic astrocytoma patients ($P = 0.23$). No association was observed between the level of GST-π expression and age in patients with lower grade gliomas ($P = 0.78$).

Correlation of the Presence of Nuclear GST-π with Tumor Histology and Patient Age. For these correlative analyses, gliomas were grouped into one of two categories, based on whether GST-π was present (Fig. 1*A*) or absent (Fig. 1*B*) in the nuclei of glioma cells. The results (Table 3) show a strong correlation ($P = 0.0003$) between the level of GST-π expression and the presence of GST-π in tumor cell nuclei. Seventy-four % of gliomas with high GST-π expression were also nuclear GST-π positive, compared with 55% of tumors with moderate and 11% with low GST-π levels. The correlation between patient age and the presence of nuclear GST-π was highly significant, with a *P* of 0.0024 by Kruskal-Wallis analysis. Seventy-nine % of the tumors of patients aged 60–75 years were nuclear GST-π positive, compared to 22% of the tumors of patients between 15 and 39 years of age. The median age of glioblastoma patients with no nuclear GST-π was 50 years (range, 49–75 years) compared with 65 years (range, 30–69 years) for those with nuclear GST-π. As shown in Table 3, no statistically significant correlation was observed between histology and the presence of nuclear GST-π in glioma cells ($P = 0.63$ by exact χ^2 analysis).

Correlation of Tumor GST-π Expression Level and Nuclear Presence with Patient Survival. Univariate and multivariate Cox proportional hazard regression models were used to examine the relationship between the level of GST-π expression and patient survival. The multivariate analyses were performed adjusting for histological grade of the tumor and patient age. The results of these analyses, summarized in Table

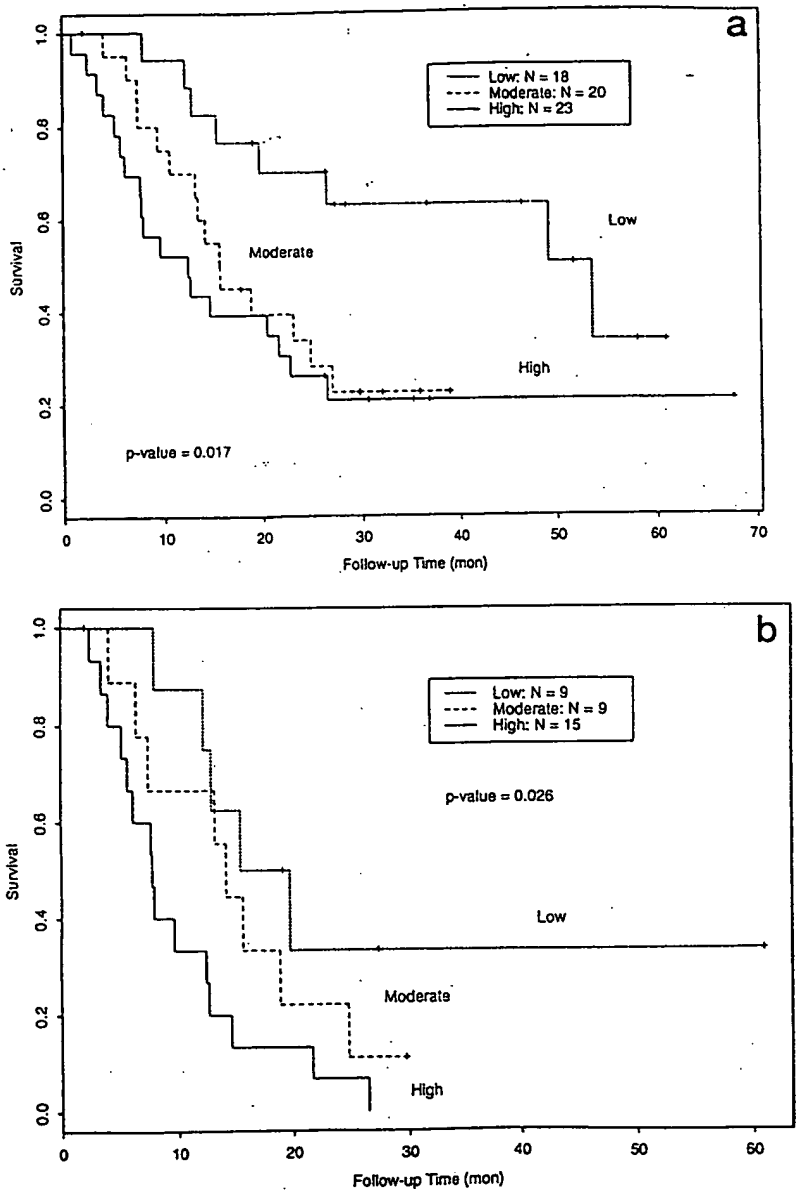

Fig. 2. Kaplan-Meier curves showing the relationship between low, moderate, and high levels of GST-π expression in malignant gliomas and patient survival. *a*, all glioma patients; *b*, glioblastoma multiforme patients.

...that patients with tumors with high (or moderate) ... levels were at a significantly higher risk of death than ... low GST-π-expressing tumors. The relative risk of ... patients with high, compared to those with low GST-π, ... CI, 1.4, 7.5; $P = 0.0069$) by univariate analysis ... CI, 1.1, 6.2; $P = 0.036$) by multivariate analysis. ... difference in survival was observed when patients with moderate and low GST-π-expressing tumors were compared, the relative risk of death associated with the presence of GST-π was 3.9 (95% CI, 2.0, 7.6; $P \leq 0.0001$) by univariate analysis and 4.4 (95% CI, 2.1, 9.2; $P = 0.0001$) by multivariate analysis.

The Kaplan-Meier survival plots for all 61 patients (Fig. 2*a*) show a strong inverse relationship between the level of GST-π expression and patient survival rate over the first 52 months of follow-up ($P = 0.017$). The difference in survival rates of patients whose tumors exhibited high or moderate GST-π expression decreased progressively with longer follow-up time. Because glioblastoma multiforme has the worst prognosis of malignant gliomas, we analyzed the subgroup of glioblastoma patients for the correlation between GST-π expression and survival. The results (Fig. 2*b*) demonstrate a significantly lower survival rate for glioblastoma patients with high GST-π-expressing tumors compared to those whose tumors expressed low or no GST-π ($P = 0.026$). Similar to the data for all 61 patients, the differences in survival of glioblastoma patients with different levels of GST-π expression was highest at the earlier stages of follow-up.

Fig. 3 shows Kaplan-Meier survival plots for the presence and absence of nuclear GST-π for all glioma patients (Fig. 3*a*) and for glioblastoma patients (Fig. 3*b*). Patients with GST-π present in the nuclei of their tumor cells had a significantly

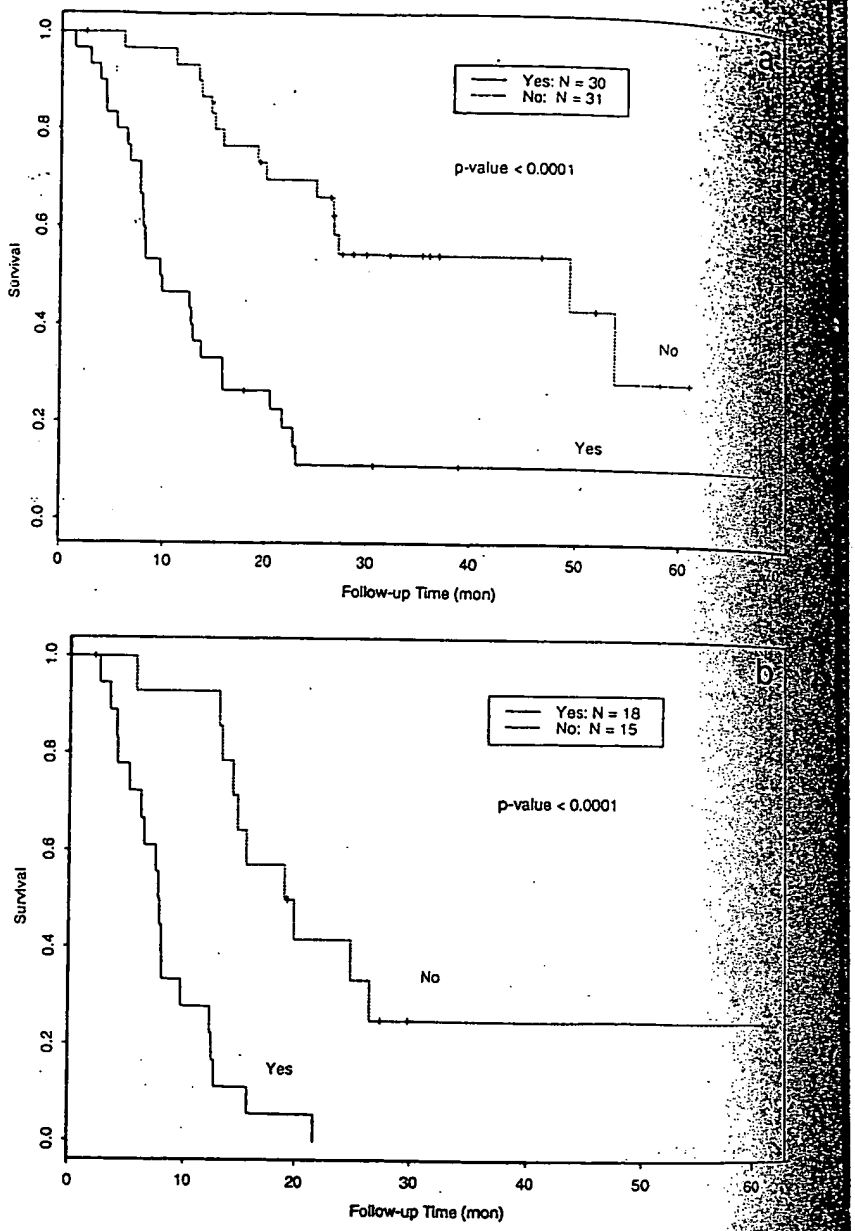

Fig. 3 Kaplan-Meier curves for the relationship between the presence and absence of nuclear GST-π in malignant gliomas and patient survival. *a*, all glioma patients; *b*, glioblastoma multiforme patients.

lower survival rate than patients whose tumor cells were negative for nuclear GST-π. For glioblastoma patients, the difference in survival was particularly strong during early follow-up. At 15 months of follow-up, approximately 92% of patients with negative nuclear GST-π tumors were alive, compared with only 3% of patients whose tumors were positive for nuclear GST-π.

DISCUSSION

The molecular mechanisms involved in the pathogenesis and malignant progression of human gliomas are still not well understood, and the most significant prognostic indicators in this disease remain the pathological and clinical parameters of histology, patient age, and Karnofsky score. There is thus a continuing urgent need for research aimed at identifying genes and gene products whose structural and functional alterations are involved in determining and maintaining the malignant phenotype and which determine therapeutic responsiveness of gliomas. Such knowledge will not only enhance diagnosis and prognosis in this disease but could also provide novel targets for the development of new and more effective and specific therapies. The present study extends our earlier observation of a correlation between the level of GST-π expression and the degree of 2-chloroethylnitrosourea resistance in glioma cell lines (40). The level of GST-π expression and its subcellular localization were analyzed in tumors of 61 glioma patients, and the results correlated with glioma histology, patient age, and patient survival. Univariate and multivariate analyses demonstrated a significant inverse relationship between the level of

GST-π expression and its presence in glioma cell nuclei with patient survival. Kaplan-Meier analyses showed that glioma patients with high or moderate GST-π-expressing tumors had a significantly lower survival rate than those with low GST-π-expressing tumors. The correlation between the level of GST-π expression and survival was most dramatic during the early phases of follow-up but decreased in the later stages of follow-up. At 48 months, 62% of all patients with low GST-π expression in their tumors were alive compared to 21% of those whose tumors expressed high GST-π levels. The multivariate Cox regression model showed that, when adjusted for age and histology, two well-established correlates of patient survival in gliomas, and omitting nuclear GST-π, patients whose tumors expressed high GST-π had an almost 3-fold higher relative risk of death compared to those with tumors with a low level of GST-π expression. The strong association between high GST-π expression and poor patient survival observed in this study for gliomas has also been reported previously for other human cancers (26–29).

The correlation between the level of GST-π expression and histology was significant within the histological categories (glioblastoma multiforme, anaplastic astrocytoma, and low-grade gliomas) but not significant when examined across the histological groups. The proportion of tumors with high GST-π expression was highest in glioblastoma multiforme and lowest in low-grade gliomas. Conversely, the highest and lowest proportions of tumors with low GST-π expression were observed among low-grade gliomas and glioblastoma multiforme, respectively. The positive association of the level of GST-π expression with histological grade of gliomas is consistent with the observations in many other human cancers (11–26). A notable exception is the prostate, in which *GST-π* gene expression has been shown to decrease with malignancy, a result of 5-methyl cytosine methylation of a CpG dinucleotide at the *Bss*HII site in a region upstream of the *GST-π* gene promoter (46).

A highly significant finding of the present study was the strong inverse correlation observed between the presence of nuclear GST-π staining and poor patient survival. Patients whose tumors expressed nuclear GST-π had a 4-fold higher relative risk of death when compared with patients whose tumors lacked nuclear GST-π. This observation suggests that nuclear GST-π localization in glioma cells is a strong prognostic indicator for this disease and is associated with a more aggressive glioma biology. The presence of nuclear GST-π in tumor cells is not unique to gliomas and has also been reported in the cells of other tumors (13, 32, 60). To our knowledge, however, this is the first study that examines the statistical correlation between nuclear GST-π and patient survival or tumor histology.

A modest, positive trend was observed between patient age and the level of GST-π expression. The relatively high median age of the patients in this study may have precluded the detection of a true age-relatedness of GST-π expression. Overall, however, the results are similar to those in a previous study of node-negative breast cancer in which a stronger correlation was observed between the level of GST-π expression and patient survival than with patient age (26). Interestingly, we observed a strong correlation between nuclear GST-π and patient age. The proportion of tumors with GST-π-positive cell nuclei increased with increasing age of the patients, from 22% in the age range of 15–39 years to 79% in the age range of 60–75 years.

Despite the significant inverse correlation between the level of GST-π expression and patient survival observed in this study, the role that the GST-π gene and its encoded protein play in malignant progression of gliomas is unclear. The best known function of the GST-π protein is that of catalysis of the conjugation of GSH with electrophiles, including carcinogens and alkylating anticancer agents (3–5), resulting in a reduction in the ability of these agents to react with DNA and other cellular macromolecules. In normal cells and tissues, the GST-catalyzed *S*-conjugation of DNA-damaging agents with GSH protects the cellular genome and decreases mutational rates and malignant transformation caused by these agents (6). In tumor cells, on the other hand, this process leads to decreased toxicity of anticancer agents, resulting in drug resistance and therapeutic failure. Although the GST-catalyzed conjugation/inactivation of electrophiles with GSH could account for the cancer preventive function of GSTs and explain their role in tumor drug resistance, such a process does not adequately explain the observed association of GST-π overexpression with higher grade tumors, disease progression, and/or decreased patient survival. These observations indicate that the *GST-π* gene and its products may be involved in tumor progression by as-yet-unidentified mechanisms. This may be related to the fact that the *GST-π* gene is clustered with several cancer-related genes and proto-oncogenes, including, *bcl1/cyclin D1, int2, hstf1, men1, and sea* (47), in a metastable region of chromosome 11q13. Abnormalities of this chromosomal region and the genes located in it, including rearrangements, translocations, coamplification, and aberrant expression, have been reported in a variety of human tumors (48–54). A number of these genes are also involved in cell cycle and cell growth regulation, and, thus, abnormalities in them could result in a deregulated cell cycle and altered cell growth, common features of malignant glioma cells. It remains to be determined whether altered or aberrant expression of the *GST-π* gene, either alone or in concert with alterations in some of these genes, may be involved in the growth and malignant progression of human glioma cells.

The presence of nuclear GST in tumor cells has potential implications for therapy in light of previous studies that demonstrated the existence of a distinct nuclear GSH pool in the cells of some tumors and cell lines (55). It is well established that GSH not only reacts directly with electrophilic anticancer agents, including cisplatin (56) and ifosphamide (57), but that it can also quench DNA cross-link precursors of cisplatin (58) and 2-chloroethylnitrosoureas (59) and possibly other bifunctional anticancer agents. This suggests that in cells with nuclear GST, catalysis of the reactions of GSH with DNA monoadducts of the agents could result in a high level of protection of the genome from damage. Nuclear GST-π-containing tumors will thus be more resistant to therapy with bifunctional alkylators than those without nuclear GST, as has been suggested for neuroblastoma (60). Further studies are, however, needed to determine whether the presence or absence of nuclear GST-π actually contributes to differential drug sensitivity in human gliomas. In light of our recent finding of allelic polymorphism in the human *GST-π* gene locus and the evidence that the different *GST-π* genes

encode functionally different GST-π proteins, it would be interesting, in future studies, to examine whether the *GST-π* genotype and GST-π phenotype has any correlation with histology, response to therapy, and/or patient survival. It would also be important to clarify, at the molecular level, the mechanisms involved in the overexpression of the *GST-π* gene in human gliomas and in the translocation of the GST-π protein into the nuclei of glioma cells, as well as whether down-regulation of the *GST-π* gene in glioma cells will alter their malignant behavior. The results of such studies will provide important insights into the role of the *GST-π* gene in the malignant process in gliomas and provide novel therapeutic approaches for this disease.

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